Docket No : D0590 70011US00
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Geert Karel Maria Plaetinck et al.
Serial No.:	10/057,108
Confirmation No.:	1549
Filed:	January 25, 2002
For:	CHARACTERISATION OF GENE FUNCTION USING DOUBLE STRANDED RNA INHIBITION
Examiner:	D. H. Shin
Art Unit:	1635

Declaration of Dr. Erwin Sablon Under 37 C.F.R. § 1.132

1. I have held the position of Director, Project Management at Ablynx NV in Gent, Belgium since October 2008. Prior to that, I was Associate Director, Project Management at Ablynx NV. Prior to that, I was Head, Infectious Diseases Dept., R&D at Innogenetics in Gent, Belgium from 2005-2008; Head, Virology Dept, R&D at Innogenetics NV from 1998 — 2005; and Head, Microbiology & bioprocessing unit at Innogenetics NV from 1996 — 1998, Group Leader microbial expression technology and protein engineering at Innogenetics NV from 1992-1996, and scientist at the Microbiology Dept., Innogenetics NV from 1990-1992. I received a Ph.D. degree in Molecular Biology from the University of Gent (Belgium) in 1990. I have published extensively and am knowledgeable in the area of molecular DNA and RNA engineering and microbial expression technologies. My curriculum vitae is attached as Exhibit 1.

2. I have reviewed the Office Action dated October 27, 2008 and it is my understanding that the claims are rejected as being obvious in view of the following combination of references: Fire et al. (U.S. Patent No 6,506,559), Zdinak et al. (Journal of Cellular Biochemistry, 1997, 67:143-153), Talkad et al. (Journal of Bacteriology, 1978, 135:528-541) and Noren et al. (U.S. Patent No. 5,691,140).

3 I am not an inventor of the above-identified patent application. I am not an author of any of the articles referenced in the Office Action in rejecting the claims, and am not an inventor of any of the patents referenced in the Office Action in rejecting the claims.

4. I have been informed that the standard used for determining whether claims are unpatentable for obviousness is that: (1) the combination of prior art references must teach all of the elements of the claimed invention; (2) a reason to combine teachings of the references must exist; and (3) there was reasonable expectation of success in obtaining the claimed invention by combining the elements from the prior art

5 The person of ordinary skill in the art is a graduate or post-graduate scientist with several years of research experience in molecular biology and microbial expression technologies, preferably in *in vitro* and *in vivo* expression techniques.

6 According to the claims of the above-identified application, the invention relates to methods for down-regulating the expression of a gene of interest in *C. elegans* by feeding *C. elegans* with a micro-organism that expresses dsRNA corresponding to the gene of interest. The microorganism comprises an expression vector that comprises a DNA sequence corresponding to the gene of interest, in which the expression vector comprises a promoter or promoters flanking said DNA sequence such that the promoter or promoters initiate transcription of said DNA sequence to produce double stranded RNA upon binding of a transcription factor to said promoter or promoters. Upon production of the dsRNA by the microorganism and feeding the microorganism to *C. elegans*, the expression of the gene of interest in *C. elegans* is downregulated

7. The Noren et al. patent describes multipurpose cloning vectors for *in vitro* generation of high specific-activity RNA probes. These vectors contain a multiple cloning site flanked by two phage RNA polymerase promoters positioned to express either strand of a DNA molecule inserted in the multiple cloning site. These flanking promoters can be the same (for example two

T7 promoters) or different (for example a T7 promoter at one side and a SP6 promoter at the other side)

8. The rationale of using the vectors described in the Noren et al. patent is that one can generate *in vitro*, using the appropriate phage RNA polymerase, either sense or antisense transcripts from the same vector.

9. Because the purpose of the vectors described in the Noren et al. patent is to generate highly specific RNA probes, it is crucial that only one strand becomes transcribed (either sense or antisense, but not both). This is brought about by one of two methods. First, one can linearize the vector with a particular restriction endonuclease that cuts between the insert and one of the flanking promoters in order to ensure transcription of only one strand. This is typically the case when both flanking promoters are of the same type. Second, for vectors in which the two flanking promoters are of different origin, one can use only one promoter-specific phage RNA polymerase in the *in vitro* transcription reaction.

10. The vectors described in the Noren et al. patent were clearly not intended to simultaneously produce transcripts from both directions.

11. In contrast to the vectors described in the Noren et al. patent, the above-identified application describes the use of bidirectional expression vectors for *in vivo* generation in *E. coli* of double stranded RNA. Such a use would not have been suggested to the person of ordinary skill in the art by the Noren et al. patent for the reasons stated above. Moreover, such type of vectors, having opposite RNA polymerase promoters, have been well known to and universally used by molecular biologists since the mid 1980s, when I was obtaining my PhD. To my knowledge, the use of this type of vector for *in vitro* production of both strands of double stranded (ds) RNA simultaneously, let alone *in vivo* production of dsRNA in a microorganism, was not even contemplated by any molecular biologist at that time, not until the present invention in the late 1990s.

12. On page 4 of the Office Action, the Examiner stated that: "Zdinak et al. teach that one can express a transgene in *C. elegans* by feeding *E. coli* that expresses the transgene. In fact, they teach that *E. coli* has been the usual food source for experimental *C. elegans* in the art." On page 6 of the Office Action, the Examiner stated that "feeding *E. coli* to *C. elegans* was an art-recognized method for expressing transgenes in the *C. elegans*."

13. I respectfully disagree with the statements of the Examiner made in the Office Action as referenced in paragraph 12. The argument "that one can express a transgene in *C. elegans* by feeding *E. coli* that express the transgene" cannot be concluded from the Zdinak et al article

14. The Zdinak et al. article describes the effect of starvation on the fate of a transgenic muscle cell expressed lacZ fusion protein in the nematode *C. elegans* by withdrawal of the food source: *E. coli*. As stated in the "MATERIALS AND METHODS" section beginning on page 144 and the "RESULTS AND DISCUSSION" section beginning on page 147, Zdinak et al. used the *Caenorhabditis elegans* strain PD55 in all of the experiments. *C. elegans* strain PD55 contains an integrated transgene, ccIs55, and this *C. elegans* strain was generated through microinjection and not by gene transfer from fed *E. coli* cells. See the references in the Zdinak paper: Fire A and Waterston RH (1992): Proper expression of myosin genes in transgenic nematodes EMBO J 8:3419-3428; Okkema PG, Harrison SW, Plunger V and Fire A (1993): Sequence requirements for myosin gene expression and regulation in *Caenorhabditis elegans* Genetics 135:385-404. Copies of these two articles are included herewith.

15. Therefore, by no means does the Zdinak et al. article give any evidence that can one express a transgene in *C. elegans* by feeding *E. coli* that express the transgene, let alone expressing in *C. elegans* double stranded RNA from an expression vector in *E. coli* or other microorganism.

16. In fact, none of the documents cited by the Examiner teach or suggest (alone or in combination) that one can express a double stranded RNA in a microorganism. Nor do these documents teach or suggest that when the microorganism is ingested by *C. elegans*, the double

stranded RNA is "liberated" from the microorganism and that the double stranded RNA has a biological effect in the cells of the *C. elegans*.

17. It is my opinion that the person of ordinary skill in the art would not have had a reasonable expectation of success, based on the cited prior art references, of practicing the invention claimed in the present application, of using the type of vectors known in the art to express double stranded RNA "inside" a microorganism, feeding such a microorganism that expresses the double stranded RNA ("inside" its cell wall) to *C. elegans*, and exerting an effect, from the inner content of the bacterium towards the cell cytoplasm of *C. elegans* cells.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issued therefrom.

Date: April 20, 2009

Erwin Sablon, Ph.D.